Kidney International, Vol. 4 (1973), p. 350–352

TECHNICAL NOTE

Quantitative assessment of fibrinolysis on isolated glomeruli

JEAN-DANIEL SRAER, JOHAN BOELAERT,
ODILE MIMOUNE, LILIANE MOREL-MAROGER and HELENA HORNYCH

Unité de Recherches de Néphrologie Normale et Pathologique de l'INSERM U64, Hôpital Tenon, Paris, France

Since its original description by Todd [1], the fibrin slide technique has been used to study the localization of fibrinolytic activity in various tissues. Our preliminary results indicate that glomerular fibrinolysis can be measured quantitatively using a preparation of isolated glomeruli.

Methods. 1) Preparation of isolated glomeruli. This technique was derived from that of Fong and Drummond [2]. Adult rats (Charles River strain) weighing approximately 120 g, were anesthetized with ether. Their kidneys were carefully removed by a mid-abdominal incision and were not flushed. The outer cortex was dissected with scissors, immediately cooled at 0° C, minced to paste-like consistency with a razor blade and suspended in 1/15 M phosphate buffer, pH 7.6 at 0° C. Centrifugation was carried out for 90 seconds with the speed-control set to give a maximal of 120 G. The supernatant was discarded and the pellet was suspended again and centrifuged. After seven repetitions of this procedure, the pellet, containing isolated glomeruli without their Bowman's capsule, was added to the fibrinogen solution.

2) Preparation of the fibrin slides. This technique was derived from that of Kwaan and Astrup [3]. Human plasminogen-rich fibrinogen containing factor XIII (Centre National de Transfusion Sanguine, Paris, France) was used at 1.5% in 1/15 M phosphate buffer, pH 7.6. Thrombin (Hoffman LaRoche, Basel, Switzerland) was diluted in saline (20 NIH units/ml) and layered upon a demarcated area of a glass slide (5 × 2.5 cm). By placing the slide vertically on blotting paper, residual thrombin was removed and a thin thrombin film was obtained. 0.2 ml of the fibrinogen solution, containing the glomerular suspension, was spread over the thrombin, thus forming a homogeneous fibrin film (160 μ thick) containing isolated glomeruli. Slides were kept horizontally during 1 minute at room temperature for clot formation and transferred into a moist chamber at 37° C for periods ranging from 30 to 120 minutes. After incubation, fibrin slides were fixed in 4% formaldehyde (pH 7) for one hour at room temperature, rinsed with water, stained with Harris hematoxylin (without acetic acid) for 15 min, again rinsed with water, mounted with Permount (Serlabo, Paris), coverslipped and sealed with nail polish.

3) Measurement of glomerular fibrinolysis. As areas of fibrinolysis around glomeruli appear as lytic defects in the fibrin film, the ratio of the diameter of the lytic area over that of the glomerular tuft (Fig. 1) was measured microscopically (with a graduated ocular) on at least 12 well-isolated glomeruli of each slide and taken as the "fibrinolytic index."

Results (Fig. 2). 1) Results with the described technique: Control group ($N=8$).[1] Glomerular fibrinolysis appeared after 22 to 28 min of incubation. The fibrinolytic index was highly correlated to incubation time ($P<0.001$) in each rat studied. The mean slope and the mean intercept of the regression lines were $0.028 \pm 0.00235\ min^{-1}$ and 0.2967 ± 0.1006, respectively. Two factor (rat and incubation time) variance analysis was performed, which disclosed no statistical difference between individual rats.

2) Effect of modification of experimental conditions. For each of these studies, the regression line fibrinolytic index versus time was plotted for individual rats and its slope calculated. The mean slope of each group was then compared by Student's t test to that obtained in the control group. *a) Modification of the fibrinogen* concentration ($N=3$). Fibrin plates were made with a fibrinogen solution using various concentrations (0.7, 3.0 and 6.0%) and compared to the control ones (concentration of 1.5%). Fibrinolysis was significantly lower at the two higher concentrations ($P<0.01$ with the 6% fibrinogen solution and $P<0.001$ with the 3% one). On the other hand, there was

Received for publication May 31, 1973;
and in revised form August 9, 1973.

[1] N = number of rats.

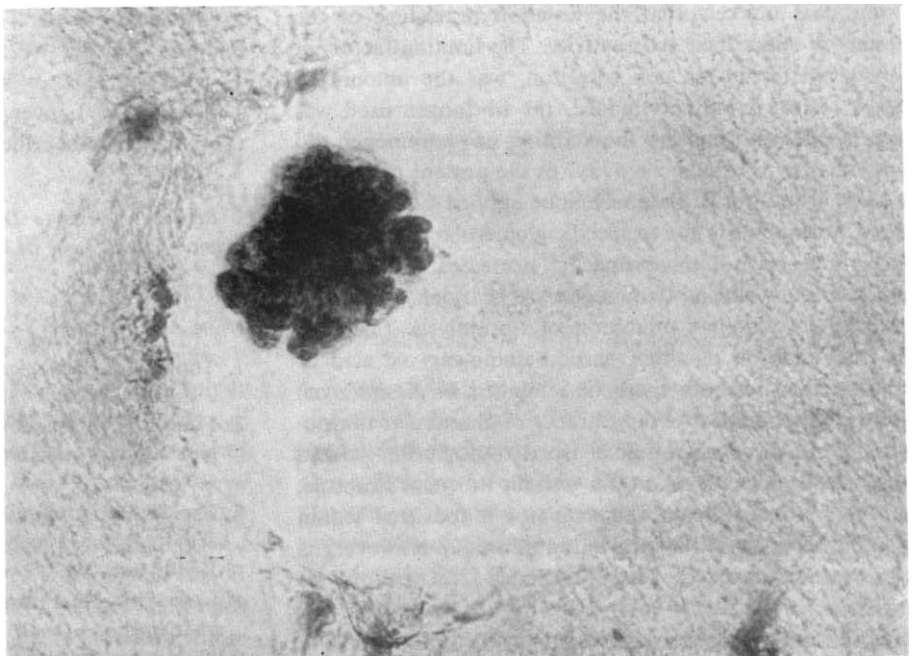

Fig. 1. *Photomicrograph of fibrinolysis around an isolated rat glomerulus after 75 min of incubation at 37° C.* (× 250).

no significant difference for the lower concentration. *b) Storage of kidneys at −20° C for 3 to 12 weeks* ($N=8$). The second kidney from each control rat was frozen immediately after removal and stored as a whole. Storage did not significantly affect fibrinolytic activity. *c) Fibrin slides without plasminogen* ($N=2$). These were prepared by spreading 0.2 ml of fibrinogen solution, before the addition of glomeruli, over the thrombin-containing slides. After clotting, the fibrin slides were maintained in a moist chamber at 80° C for 1 hour [4]. Approximately 0.1 ml of the suspension of isolated glomeruli was then spread over the fibrin film, and the slide was incubated at 37° C for as long as 180 min. No fibrinolysis could be observed. Layering the glomeruli over the surface of the plasminogen-containing fibrin slide or incorporating them within the film yielded no difference in fibrinolytic activity. *d) Enrichment of fibrinogen by addition of human plasminogen* ($N=3$). Plasminogen (Centre National de Transfusion Sanguine, Paris) was added to the fibrinogen solution at concentration of 0.1, 0.2 and 0.4 mg per ml. At these concentrations, fibrinolysis was not significantly modified. *e) Inhibition of glomerular fibrinolysis by synthetic inhibitors of plasminogen activators: Epsilon-amino-caproic acid and tranexamic acid* ($N=4$). *In vitro* addition of epsilon-amino-caproic acid 10^{-3} M (Hemocaprol, Delagrange, Paris) ($N=2$) and of tranexamic acid 10^{-4} M (Frenolyse, Specia, Paris) ($N=2$) to the fibrinogen solution resulted in a total inhibition of fibrinolysis for an incubation period up to 120 min.

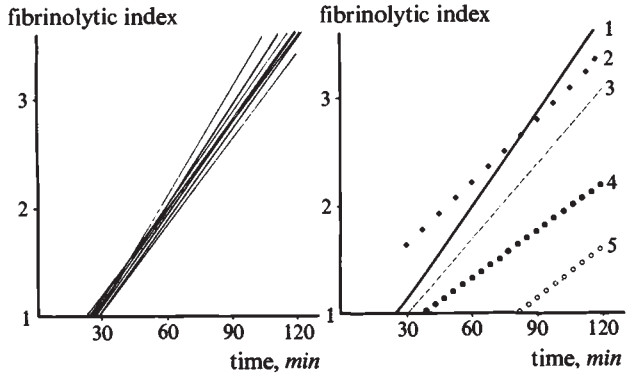

Fig. 2. "*Fibrinolytic index*" *versus incubation time. Left:* each slope corresponds to an individual "control" rat; *Right:* mean slope in various experimental conditions: *1)* control, *2)* storage at −20° C, *3, 4 and 5)* fibrinogen concentrations of 0.7, 3 and 6%, respectively.

Discussion. The method described here attempts to investigate glomerular fibrinolysis on a quantitative basis. When studied by the usual fibrin plate technique applied on kidney tissue sections, fibrinolysis appears earlier around blood vessels than around glomeruli [5]. Accurate measurement of glomerular fibrinolysis is thus difficult because more active areas in the section produce confluent zones of lysis after brief periods of incubation. As a purified suspension of isolated glomeruli was used in the present study, our technique enables glomerular fibrinolysis to be separated from that of other renal structures, mainly vascular and tubular [5, 6]. Whereas the usual fibrin plate technique, applied on renal tissue sections, appreciates either "focal lysis time" qualitatively [3] or, more recently, semi-quantitatively [7], the present method yields quantitative, highly reproducible results. Glomerular fibrinolysis appeared at 22 to 28 min after starting the incubation at 37° C. As a similar time is reported [6] using whole kidney tissue sec-

tions, it is unlikely that the isolation procedure of the glomeruli alters their lytic activity. The limiting factor, at any given fibrinogen concentration, was the amount of tissue activator present: indeed, the fibrinogen used was rich in plasminogen, and the addition of plasminogen did not enhance fibrinolytic activity. In the present method as well as in the fibrin plate technique applied on tissue sections, fibrinolysis is due to specific glomerular plasminogen activators and not to nonspecific proteases. Indeed, the use of fibrin in which plasminogen was heat-denaturated [4] resulted in complete inhibition of fibrinolysis. Likewise, *in vitro* addition of either epsilon-amino-caproic acid or tranexamic acid, both synthetic inhibitors of plasminogen activation, also led to disappearance of glomerular fibrinolysis. Since the preparation of isolated glomeruli yields a suspension of glomerular tufts without Bowman's capsule, the glomerular plasminogen activator is localized within the tuft, as suggested by previous authors [6]. However, its more precise localization at the cellular level was not yet possible. Kidney storage at −20° C up to 12 weeks did not alter fibrinolytic activity: this finding confirms the results of previous studies [3, 6]; moreover, it emphasizes the reproducibility of our quantitative method, as renal tissue from the same animal was examined at different times.

Previous qualitative studies in man and animals have attempted to evaluate the fibrinolysis of renal tissue in normal as well as in pathological conditions. This new quantitative method should allow more accurate studies of pathological glomerular fibrinolysis.

Acknowledgments. This study was supported by a grant from D.G.R.S.T. (72.70822). The authors wish to thank Dr. Larrieu, Dr. J. Vermylen (Akademisch Ziekenhuis, University of Leuven, Belgium) and Prof. R. Ardaillou (Paris) for helpful criticism and review of the manuscript.

Reprint requests to Dr. J.-D. Sraer, Service de Néphrologie, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France.

References

1. Todd AS: Histological localization of fibrinolysis activator. *J Pathol Bacteriol* 78:281–283, 1959
2. Fong SJC, Drummond KN: Method for preparation of glomeruli for metabolic studies. *J Lab Clin Med* 71:1034–1039, 1968
3. Kwaan HC, Astrup T: Demonstration of cellular fibrinolytic activity by the histochemical fibrin slide technique. *Lab Invest* 17:140–145, 1967
4. Lassen M: Heat denaturation of plasminogen with fibrin plate method. *Acta Physiol Scand* 27:371–375, 1953
5. Myrhe-Jensen O: Localization of fibrinolytic activity in the kidney and urinary tract of rats and rabbits. *Lab Invest* 25: 403–411, 1971
6. McConnell D, Johnson JG, Young I, Holemans R: Localization of plasminogen activator in kidney tissue. *Lab Invest* 15:980–986, 1966
7. Pandolfi M, Bjernstad A, Nilsson IM: Technical remarks on the microscopical demonstration of tissue plasminogen activator. *Thromb Diath Haem* 27:88–98, 1972